

What is claimed is:

1. A method of selecting a protein variant having reduced immunogenicity as compared with a parent protein, comprising the steps
5 of:

screening a random peptide display package library with antibodies raised against any protein of interest;

sequencing the amino acid sequence of the antibody binding peptides, or the DNA sequence encoding the antibody binding peptides;

10 identifying epitope patterns by sequence alignment of the reactive peptide sequence;

localization of epitope patterns on the primary and 3-dimensional structure of the parent protein;

15 defining an epitope area including amino acids situated within 5 Å from the epitope amino acids;

changing the localized epitope patterns, or amino acids defining the epitope area of the parent protein by genetic engineering mutations of a DNA sequence encoding the parent protein without impairing functionality of the protein using the emerging epitope
20 database for eliminating amino acid substitutions creating new or duplicating existing epitope patterns;

introducing the mutated DNA sequence into a suitable host, culturing the host and expressing the protein variant; and

25 evaluating the immunogenicity of the protein variant using the parent protein as reference.

2. The method of claim 1, wherein the protein variant has reduced allergenicity.

30 3. The method of claim 1, wherein the protein variants are selected by evaluation of antigenicity and/or functionality prior to the evaluation of allergenicity.

4. The method of claim 1, wherein antibodies raised against the
35 parent protein are used for screening the random peptide display package library.

5. The method of claim 1, wherein the epitope patterns of a parent protein are identified by comparison of the sequences of the peptide with the sequence and 3-dimensional structure of the parent protein.

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6. The method of claim 1, wherein the epitope area of the parent protein is identified by comparing the sequences of the peptides with the sequence and 3-dimensional structure of the protein.

10 7. The method of claim 1, wherein the parent protein is an enzyme.

8. The method of claim 7, wherein the enzyme is selected from the group consisting of glycosyl hydrolases, carbohydrases, peroxidases, proteases, lipases, phytases, polysaccharide lyases, oxidoreductases, 15 transglutaminases and glycoisomerases.

9. The method of claim 1, wherein the antibodies are IgG or IgE antibodies.

20 10. The method of claim 1, wherein the peptide display package library is a phage display library.

11. The method of claim 1, wherein the peptides of the peptide display package library are oligopeptides having from 5 to 25 amino 25 acids.

12. The method of claim 1, wherein the epitope area is changed by substituting at least one amino acid of the epitope area.

30 13. The method of claim 1, wherein the epitope area is changed by adding or deleting at least one amino acid of the epitope area.

14. The method of claim 1, wherein the epitope pattern is changed by substituting at least one amino acid of the epitope pattern.

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15. The method of claim 1, wherein the epitope pattern is changed by adding or deleting at least one amino acid of the epitope pattern.

16. The method of claim 1, wherein amino acids in the epitope area
5 are changed by substituting and/or inserting at least one amino acid by an amino acid which renders the substituted and/or inserted amino acid a target for covalent conjugation to an activated molecule.

17. The method of claim 16, wherein the amino acid for substitution
10 and/or insertion is selected from the group consisting of K, R, C, D, E.

18. The method of claim 16, wherein the molecule for covalent
conjugation is selected from the group of activated synthetic or
15 natural polymers.

19. The method of claim 18, wherein the activated synthetic polymer is a polyethylene glycol.

20. The method of claim 1, wherein the immunogenicity is measured by competitive ELISA.

21. The method of claim 20, wherein the immunogenicity of the protein variant is below 75% of the immunogenicity of the parent protein.

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22. A protein variant having reduced immunogenicity as compared with its wild-type protein, wherein the amino acid sequence of the protein variant differs from the amino acid sequence of the parent protein with respect to at least one epitope area of the parent protein, such
30 that the immunogenicity of the protein variant is below 75% of the immunogenicity of the parent protein.

23. The protein variant of claim 22, wherein the epitope areas correspond to epitope patterns that correspond to reactive peptide
35 sequences reactive to antibodies raised against the wild-type protein.

24. The protein variant of claim 22, wherein the epitope pattern is an IgE epitope pattern.

25. The protein variant of claim 22, wherein an anchor amino acid of
5 the epitope is substituted or deleted.

26. The protein variant of claim 22, wherein the immunogenicity is below 50% of the immunogenicity of the parent protein.

10 27. The protein variant of claim 22, wherein the epitope areas are defined on the wild-type protein structure by being localized less than 5 Angstroms from any of the following epitope patterns: R Y P R/K, S G P R A G, P R/K S D P G, D P > R D T G, A R > R > A > N, N N > E L, R/K R F A/S N > E/D, E Y > M, P > P A P > S, A K I D P R/K, A D S
15 > G Y P, S R S A, L > G R S S.

28. A composition comprising a protein variant of claim 22.

29. A DNA construct comprising a DNA sequence encoding a protein
20 variant of claim 22.

30. An expression vector comprising a DNA construct of claim 29.

31. A host cell which is capable of expressing a polypeptide and
25 comprises a DNA construct of claim 29.

32. A host cell which is capable of expressing a polypeptide and comprises an expression vector of claim 30.

30 33. The host cell of claim 31, which is a fungal cell, an insect cell, a mammalian cell, or a plant cell.

34. A method of producing a protein variant having reduced immunogenicity as compared with the parent protein, comprising:

culturing a host according to claim 31 in a suitable culture medium to obtain expression and secretion of the protein into the medium, followed by

isolation of the protein from the culture medium.

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